

35. (Amended) A modified pneumolysin polypeptide, wherein the polypeptide is partially-soluble, has attenuated hemolytic activity, and wherein the modification of the polypeptide comprises an amino acid sequence having SEQ ID NO:3 modified to possess ^{at} (at least one amino acid substitution selected from the group consisting of residues 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257.

53. (Amended) The polypeptide according to claim 35, wherein the polypeptide is obtained by randomly mutating a nucleic acid molecule encoding a pneumolysin polypeptide.

60. (Amended) The polypeptide according to claim 35, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.

62. (Amended) The vaccine comprising the polypeptide according to claim 35 and a pharmaceutically acceptable carrier.

64. (Amended) The vaccine according to claim 63, wherein the polysaccharide is a bacterial polysaccharide and is from a bacterium selected from the group consisting of *Haemophilus influenzae* type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus type Ia, Ib, II, III, V, or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.

65. (Additional) The polypeptide according to claim 35, wherein at least one amino acid substitution is a proline or hydroxyproline at residue 61.

66. (Additional) The polypeptide according to claim 35, wherein at least one amino acid substitution is a ^{lysine}lysine, ^{arginine}arginine or ^{histidine}histidine at residue 148.

67. (Additional) The polypeptide according to claim 35, wherein at least one amino acid substitution is a ^{leucine}leucine, ^{glycine}glycine, ^{alanine}alanine, ^{valine}valine or ^{isoleucine}isoleucine at residue 195.

68. (Additional) The polypeptide according to claim 35, wherein at least one amino acid substitution is an ^{arginine}arginine, ^{valine}valine, ^{glutamic acid}glutamic acid, or ^{serine}serine at residue 243.

69. (Additional) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at residues 17, 18, 61, 66 and 101.

70. (Additional) The polypeptide according to claim 69, wherein the substitutions consist of arginine for residue 17, asparagine for residue 18, proline for residue 61, tyrosine for residue 66, and threonine for residue 101.

71. (Additional) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at residues 41, 172, 195 and 255.

72. (Additional) The polypeptide according to claim 71, wherein the substitutions consist of glycine for residue 41, alanine for residue 172, isoleucine for residue 195, and glycine for residue 255.

73. (Additional) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at residues 63, 127, 128 and 148.

74. (Additional) The polypeptide according to claim 73, wherein the substitutions consist of serine for residue 63, glutamic acid for residue 127, histidine for residue 128, and lysine for residue 148.

Sub G5 75. (Additional) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at residues 33, 46, 83, 239 and 257.

Flb cont 76. (Additional) The polypeptide according to claim 75, wherein the substitutions consist of threonine for residue 33, threonine for residue 46, serine for residue 83, arginine for residue 239 and glycine for residue 257.

77. (Additional) The polypeptide according to claim 75, wherein the substitutions at residues 33, 46 and 83 are either a serine, threonine, asparagine, glutamine, tyrosine or cysteine; the substitutions at residue 239 is either a lysine, arginine or histidine; and the substitution at residue 257 is either a leucine, glycine, alanine, isoleucine or valine. new

78. (Additional) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at residues 45, 102, 189 and 195.